Proc. Natl. Acad. Sci. USA
Vol. 77, No. 1, pp. 604–608, January 1980
Medical Sciences

Association of coronary atherosclerosis with hyper*apo*betalipoproteinemia [increased protein but normal cholesterol levels in human plasma low density (β) lipoproteins]

(coronary artery disease/hyperlipidemia)

ALLAN SNIDERMAN*, STANLEY SHAPIRO*, DEREK MARPOLE*, BERNARD SKINNER*, BABIE TENG*, AND PETER O. KWITEROVICH, JR.†

* Cardiovascular Research Unit, Royal Victoria Hospital and the Department of Epidemiology, McGill University, Montreal, Canada; and †Departments of Pediatrics and Medicine, Lipid Research Clinic, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Communicated by Victor A. McKusick, October 29, 1979

ABSTRACT **Most patients with coronary artery disease do not have elevated plasma or low density lipoprotein (LDL) cholesterol. To test whether the protein moiety of LDL, LDL B, might be a parameter to identify ischemic heart disease, the plasma cholesterol, triglyceride, LDL cholesterol, and LDL B were measured in 100 consecutive patients undergoing cardiac catheterization. On the basis of coronary angiography, these patients were divided into two groups: group I, 31 patients without, and group II, 59 patients with significant coronary artery disease. Although cholesterol, triglyceride, and LDL cholesterol levels were all significantly higher in group II, discriminant analysis indicated that LDL B concentrations most clearly separated the two groups. In group I (noncoronary), LDL B was 82 ± 22 mg/100 ml, whereas in group II, LDL B was 118 ± 22 mg/100 ml. The B protein level in group I was similar to other normal groups studied (35 asymptomatic male physicians, 83 ± 11 mg/100 ml; 90 normolipidemic medical students, 72 ± 17 mg/100 ml). The results therefore indicate that not only does LDL B better separate coronary and noncoronary groups than other lipid parameters studied, but also, among those with coronary artery disease, there exists a group with normal LDL cholesterol but with levels of LDL B protein similar to those observed in type II hyperlipoproteinemia. The explanation for the altered LDL composition observed in this group remains to be elucidated.**

Atherosclerotic coronary artery disease is a major cause of morbidity and mortality in industrialized countries. The importance of plasma levels of certain lipids and lipoproteins in the pathogenesis of atherosclerosis has been supported by a number of epidemiologic and genetic studies. Briefly, increased levels of total plasma cholesterol and the major cholesterol-carrying lipoproteins, low density (β) lipoproteins (LDL), are associated with an increased risk of developing coronary artery disease (1, 2). Whether an elevated level of plasma triglycerides is an independent risk factor for coronary artery disease is controversial; conflicting evidence has been presented in both epidemiological surveys (3, 4) and in studies in kindreds with familial hypertriglyceridemia (5, 6). A low concentration of another lipoprotein, high density (α) lipoproteins (HDL), appears to be a separate risk factor for coronary artery disease (7), whereas high levels of HDL may have a protective effect (8).

Many patients who develop coronary artery disease have normal plasma lipid and lipoprotein cholesterol levels. The presence of disease in some of these patients may be related to the effect of other risk factors such as hypertension and cigarette smoking. Nevertheless, the presence of coronary artery disease in a significant number of these patients remains unexplained.

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A high correlation between total plasma and LDL cholesterol levels has been found, and it has been assumed that the measurement of LDL by determining its cholesterol content provided an accurate assessment of the concentration of the total LDL particle (9). This may be true in general but may not hold in all patients. The present study focuses upon the measurement of the protein moiety of LDL, LDL B, in patients with and without coronary artery disease. Several control groups, as well as a group with type II hyperlipoproteinemia (10), are included for comparison. The objectives of the study were: (*i*) to determine if measurement of LDL B discriminates cases of coronary artery disease better than measurement of LDL cholesterol, total plasma cholesterol, and triglycerides; and (*ii*) to determine whether there may be a subset of patients with coronary artery disease with normal total plasma cholesterol levels who have elevated LDL B but normal LDL cholesterol levels.

METHODS

During a 3-month period, plasma samples were collected from 100 consecutive patients undergoing elective diagnostic coronary angiography at the Royal Victoria Hospital. These studies were carried out either as a presurgical evaluation for valvular or ischemic heart disease or, alternatively, for diagnosis of atypical chest pain or unexplained heart failure. None of these patients was receiving lipid-lowering medication. However, a systematic evaluation of their diet was not obtained. The decisions for, and timing of, the catheterization were made by physicians not involved in this study. In all cases, left ventriculography and cineangiography were done by the Seldinger technique and the right and left coronary arteries were visualized in at least two projections. All coronary arteriograms were read "blind" by a cardiologist and a radiologist independently.

Patients were judged "free of coronary disease" in that no coronary vessel had more than minimal involvement (maximum of 25% stenosis). Subjects were judged to have coronary artery disease based on the finding of a greater than 50% stenosis of a major coronary artery. With these criteria, there was no disagreement between the two observers. Angiograms were not matched to lipoprotein values until after completion of analysis of the entire 100 patients.

Lipoprotein Analysis. Blood was collected in tubes containing 1 mg of EDTA per ml; plasma was then separated by centrifugation at 2500 × *g* at 4°C. LDL was isolated by ultracentrifugation (11). A 50 Ti rotor was used in a Beckman L5-

Abbreviations: LDL, low density (β) lipoproteins; HDL, high density (α) lipoproteins; VLDL, very low density (pre-β) lipoproteins; IDL, intermediate density lipoproteins.

65B ultracentrifuge. The initial sample was ultracentrifuged for 18 hr at 105,000 × *g*. The supernate of density 1.006 g/ml was removed by pasteur pipette and the density of the infranate was raised to 1.063 g/ml by addition of NaCl/KBr. After the ultracentrifugation was repeated, the supernate was again removed by pasteur pipette; this supernate constituted LDL, density 1.006–1.063 g/ml.

Cholesterol was determined both in plasma and LDL by the method of Abell *et al.* (12), with the color reagent of Zak *et al.* (13). Plasma triglyceride was determined according to Carlson (14); LDL B protein was determined by radial immunodiffusion (15) in plasma and again in the supernate with density 1.006–1.063 g/ml. LDL recovery could then be determined as:

$$\% \text{ LDL recovery} = \frac{\text{mg B protein in LDL supernate}}{\text{mg LDL B protein in plasma sample}} \times 100.$$

Except in two cases, the lowest recovery was 92% and the mean (excluding these two) was 95%. B protein is present in very low density lipoproteins (VLDL) and in intermediate density lipoproteins (IDL) as well as in LDL. The assay used in this study was designed so that VLDL B protein would not interfer with the estimate of LDL B protein. Because of its larger size, VLDL does not migrate into the 1.5% agarose gel with sufficient speed to participate in the immune reaction within the 18-hr incubation period. This conclusion is based on experiments in which very large amounts of VLDL (up to 160 mg/100 ml of protein) added to small amounts of LDL (34 mg/100 ml of B protein) did not change measured LDL B protein (15). LDL B protein can be measured by use of a plasma sample, but was isolated by ultracentrifugation in this study so that its cholesterol content could be measured and its composition then estimated.

Patient Exclusions. Ten patients were eliminated after completion of both the lipid measurement and analysis of the coronary arteriograms. In three patients, lesions of between 25 and 50% obstruction were noted. These patients were eliminated because the clinical significance of such lesions is not known. Five patients, two without and three with significant coronary disease, had type II hyperlipoproteinemia (hyperbetalipoproteinemia), which was defined as an LDL cholesterol greater than 200 mg/100 ml (10). In two cases, there was poor recovery of LDL after ultracentrifugation. This artificially low LDL cholesterol would bias the data. The LDL B protein measured in plasma in these two patients was 91 and 128 mg/100 ml, respectively, but in each case less than 70% of the protein was recovered in the supernate of density 1.063 g/ml (the remainder was in the infranate). Both of these patients had coronary artery disease.

In addition to the above, data were collected on several additional groups. Group A consisted of 30 patients (18 men and 12 women, age 49 ± 5 years) with atypical chest pain and normal coronary angiograms studied either before or after the 3-month trial; group B consisted of 35 male physicians, mean age 53 ± 6 years, none of whom had symptoms of ischemic heart disease; group C consisted of 90 normolipidemic medical students; and group D consisted of 40 patients with type II hyperlipoproteinemia. In the last two groups, total and LDL cholesterol were determined at the Lipid Research Clinic, Johns Hopkins Hospital (16). Aliquots of fresh plasma samples from groups C and D were frozen at −20°C until shipment on dry ice by air to the Royal Victoria Hospital. Plasma LDL B protein was then measured by radial immunodiffusion (15). The value of LDL B is unaffected by a single freezing of plasma (unpublished data).

Table 1. Plasma lipid and lipoprotein levels (mg/100 ml) in patients without (group I) and with (group II) coronary artery disease

Group	Total cholesterol	Total triglyceride	LDL cholesterol	LDL B protein
I	168 (27)	99 (46)	112 (30)	82 (15)
II	209 (37)	149 (71)	134 (27)	118 (22)

Values are given as mean (±SD).

Statistical Methods. Logarithmic transformations were carried out on the lipid measurements and statistical analyses were performed in parallel—i.e., each analysis was carried out on both the transformed and untransformed data. In all cases, the results of these parallel analyses were consistent. The data are reported in their untransformed mode.

Student's *t* tests were used to examine the question of differences in mean lipid values between the coronary and noncoronary groups. Stepwise discriminant analyses (17) were performed in order to examine the separation of the two groups attributable to B protein. The numerical accuracy of the computations was checked by using two different computer programs, BMDP 7M (18) and SPSS DISCRIMINANT (19), to execute the discriminant procedure.

RESULTS

Of the 90 patients who underwent coronary angiography, 31 did not have significant coronary artery disease and constitute group I (17 men and 14 women, mean age 47.1 ± 14.7 years). Twenty had either aortic or mitral valve disease; two had cardiomyopathy; in nine studied because of chest pain, no cardiac abnormalities were discovered. Fifty-nine patients had at least one coronary artery with greater than 50% obstruction and constituted group II (48 men and 11 women, mean age 53.9 ± 9.0 years).

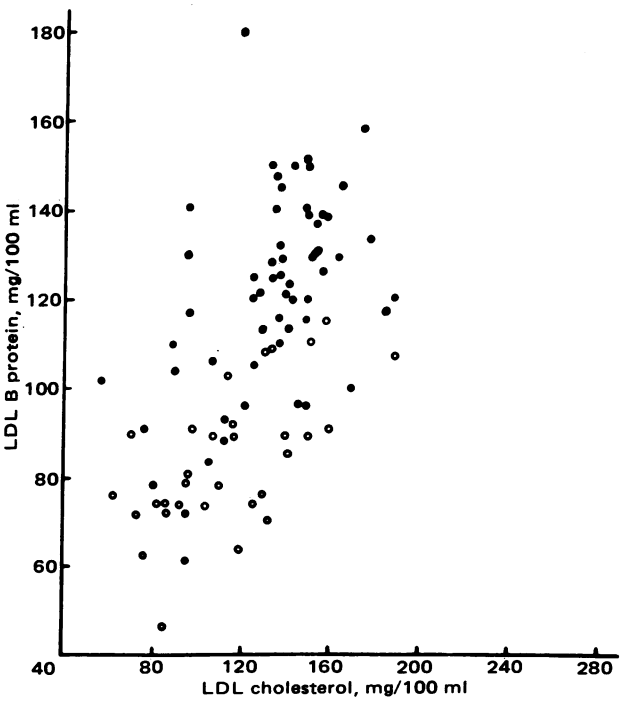

FIG. 1. LDL cholesterol and B protein levels are shown for each patient in groups I and II. O, Group I (noncoronary patients); ●, group II (patients with coronary artery disease). Although there is marked overlap between the two groups for LDL cholesterol, there is better separation by LDL B protein.

Table 2. Plasma LDL cholesterol and B protein levels (mg/100 ml) in groups A–D

Group	*n*	LDL cholesterol	LDL B protein
A	30	114 (25)	84 (13)
B	35	118 (15)	83 (11)
C	90	102 (28)	72 (17)
D	40	250 (55)	138 (20)

Values are given as mean (±SD). Group A, patients (18 men, 12 women) with mean age of 49 years with atypical chest pain and normal coronary arteries by angiography. Group B, male physicians with mean age of 53 years without clinical evidence of ischemic heart disease. Group C, normolipidemic medical students. Group D, patients with type II hyperlipoproteinemia (10).

The difference in means for each lipid variable between the coronary and noncoronary groups was statistically significant at or below the 0.05 level (Table 1). The ability of each variable to separate groups I and II was tested by stepwise discriminant analysis. This procedure selectively adds and deletes variables in order to achieve a discriminant function that best separates the two groups. To adjust for differences in the sex and age composition of the groups, we entered these two items first to determine and then correct for any influence. Subsequently, the program selected in turn that variable that best added to the separation of the groups. The first chosen was LDL B protein ($P < 0.001$). However, once B protein entered, no other individual lipid parameter provided a significant additional contribution to the total variance.

In order to examine the residual effect of B protein, we performed subsequent analyses with the constraint that B protein was the last variable available for entry. Thus, its ability to enter the discriminant would be reduced to the extent that the other variables (i.e., total cholesterol, LDL cholesterol, and plasma triglycerides) could either account for the variability between groups or provide the same information as contained in B protein. Even after the effect of the other variables had been accounted for, the effect of B protein remained significant ($P < 0.001$), indicating that LDL B provided information not present when the other variables were considered alone or in combination. These analyses were repeated comparing only members of the same sex in groups I and II without altering the results.

The ability of LDL cholesterol compared with LDL B to separate groups I and II can be seen (Fig. 1). Although the mean

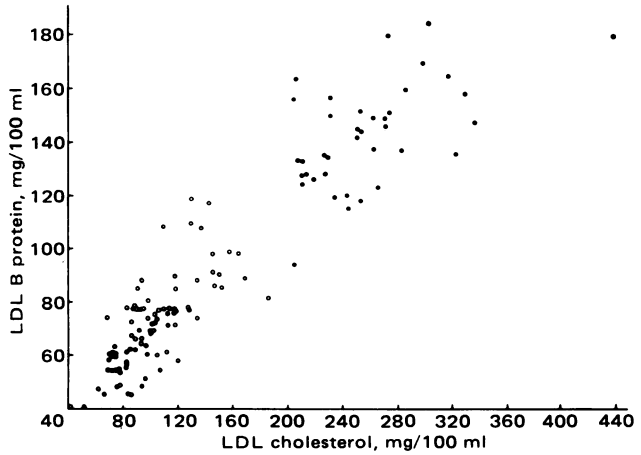

FIG. 2. LDL cholesterol and B protein values are shown for 90 medical students (O, group C) and patients with type II hyperlipoproteinemia (10) (●, group D). Patients with type II hyperlipoproteinemia have both increased LDL cholesterol and B protein levels.

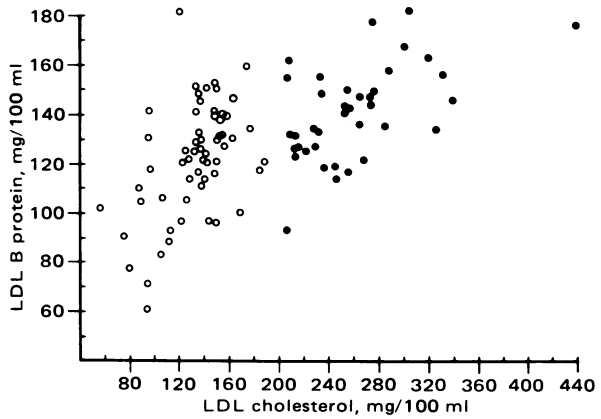

FIG. 3. Data for plasma LDL cholesterol and B protein are compared between patients with coronary disease (O, group II) and those with type II hyperlipoproteinemia (10) (●, group D). Although the LDL cholesterol values are lower in the coronary group, many of the patients with coronary disease have LDL B protein levels similar to those in patients with type II hyperlipoproteinemia.

LDL cholesterol in group II was higher than in group I (134 ± 27 mg/100 ml compared to 112 ± 30 mg/100 ml), there was very extensive overlap. LDL B was also higher in group II than in group I (118 ± 22 mg/100 ml compared to 82 ± 15 mg/100 ml). For LDL B, however, there was considerably less overlap between those without coronary disease (group I) and those with coronary disease (group II).

Because there is much less information about LDL B levels than about LDL cholesterol levels, the results of LDL cholesterol and B protein for groups A–D were included and are given in Table 2. Group A consists of 30 individuals with normal coronary arteries; group B, 35 asymptomatic male physicians; and group C, 90 medical students. The means for LDL B in these three groups were 84 ± 13, 83 ± 11, and 72 ± 17 mg/100 ml, respectively. These values all closely resemble those obtained in group I (noncoronary disease).

In contrast, the LDL B in group D (40 patients with type II hyperlipoproteinemia) was elevated: 138 ± 20 mg/100 ml. The LDL cholesterol and B protein levels from groups C and D were plotted (Fig. 2). As anticipated, patients with type II hyperlipoproteinemia have both LDL cholesterol and LDL B protein elevated above normal and fall in the right upper sector, whereas the normolipidemic controls (group C) are in the lower left sector.

The results from group II and group D were plotted (Fig. 3). Coronary patients (group II) are located primarily in the upper left sector, with high LDL B but normal LDL cholesterol. Patients with type II hyperlipoproteinemia (group D) are clustered in the upper right sector, with high levels of both LDL B and LDL cholesterol. Therefore, patients in group II had, in general, elevated levels of LDL B comparable to these patients with type II hyperlipoproteinemia, but they had considerably lower levels of LDL cholesterol.

DISCUSSION

This study examined the concentrations of the cholesterol and protein moieties of LDL in 100 consecutive patients undergoing coronary arteriography. The most important findings in this study are related to the power of LDL B to discriminate between those with and those without coronary atherosclerosis and to the tentative identification of a subgroup of patients who have high levels of LDL B but normal concentrations of LDL cholesterol. We have chosen to term this group hyper*apo*betalipoproteinemia to distinguish them from patients with hyperbetalipoproteinemia (type II hyperlipoproteinemia) (10).

The diagnosis of coronary atherosclerosis, or its absence, was made by coronary arteriography. The patients were consecutive and unselected and the study was blind until completed. The observations made in this cross-sectional study are not directly comparable with longitudinal studies made in general populations. Those with coronary disease (group II) are representative of our population of diseased patients. For example, there are considerably more men than women within this group. Because consecutive patients were entered, this outcome is expected because symptomatic coronary disease is more frequent in men. However, a direct consequence of the study design was that the control group (group I) without coronary atherosclerosis is not truly a "normal" population because many had other types of nonatherosclerotic cardiovascular disease. Further, the two groups (I and II) had different age and sex characteristics.

The LDL B protein levels in the control group (group I) may be compared with those previously found by others in normal populations, as well as with the other normal groups (A–C) studied by us. LDL B protein levels in normal subjects have been measured by several groups by using variants of radioimmunoassay techniques. Although the measurement technique differs, the results reported are in reasonable agreement with each other and those now reported (20–26). For example, in a cross-sectional study, Albers *et al.* (20) showed that in normal women the LDL B protein level increases by 12 mg/100 ml from the third to the sixth decade (75 ± 18 to 86 ± 16 mg/100 ml), whereas in normal men, the rise is only 4 mg/100 ml (81 ± 18 mg/100 ml). An increase of only 10% of LDL B protein was also noted by Hueck *et al.* (23) over the 30-year period from 30 to 60 years of age. The study of Bedford and coworkers (24) failed to show any significant sex differences for LDL B protein. The data from our secondary groups (A–C) provide additional information from our own laboratory. The similarity of B protein levels in a further 30 patients with atypical chest pain and normal coronaries (group A, LDL B = 84 ± 13 mg/100 ml) to those of the primary control group indicates that LDL B protein levels in the latter were not altered by either rheumatic heart disease or cardiomyopathy. Similarly, the primary control group does not differ significantly from the LDL B protein of 83 ± 11 mg/100 ml found in the group of male physicians whose mean age was 53 years.

Thus, the level of LDL B protein in the primary control group corresponds to that in other groups of normal subjects tested not only in our laboratory, but in others, and so we conclude that not only did the control group have normal coronary arteries, but they also had normal levels of LDL B protein. We have indicated that there is some evidence that age and sex do influence LDL B protein. This evidence also indicates that such influence is slight and, even if uncorrected, could not produce the observed differences between the experimental and control groups. Even so, the statistical analysis in the present study was not conducted on uncorrected data, but only after due allowance was first made for any age and sex difference between the two primary groups.

In both groups I and II, B protein was measured both in plasma and in the fraction of plasma with density 1.006–1.063 g/ml isolated by ultracentrifugation. VLDL, the other major lipoprotein class, which contains apoB, should not be present in this density range. However, even if contamination with VLDL had occurred during isolation, VLDL B is not measured by this assay (15). In normal plasma, small amounts of an IDL (density 1.006–1.019 g/ml) would be present in the fraction of density 1.006–1.063 g/ml. We have previously investigated the amount of apoB in IDL in a similar group of patients and found that it contributed minimally to the B protein in the 1.006–1.063 g/ml range (27). Further, we have measured plasma LDL B levels in 20 patients with type III hyperlipoproteinemia, an inherited defect characterized by the presence of relatively large amounts of IDL (10), and have not observed elevated levels of plasma LDL B [mean (±SD) = 57(19) mg/100 ml]. Therefore, the difference in LDL B levels between groups I and II is unlikely to be due to contamination with either VLDL or IDL.

This study is consistent with much previous work indicating that total plasma and LDL cholesterol and total triglycerides are all significantly higher in groups of patients with coronary disease when compared to those without atherosclerosis. In this study, LDL B protein discriminated better than the other parameters listed above. This conclusion was based on discriminant analysis. A highly significant result was obtained whether LDL B was free to enter as the first variable (in which case no other variable provided any additional separation) or whether the effect of the other variables had first been taken into account (in which case LDL B then provided further significant separation). The importance of LDL B can be appreciated further by the significant clustering of patients with high LDL B but normal LDL cholesterol who had coronary artery disease (Fig. 1). The data therefore also suggest the presence of a subgroup of patients with coronary disease who have an abnormally low ratio of cholesterol to B protein in LDL when compared to the control groups (groups I and A–C). These observations expand the earlier report of Avogaro and coworkers (28), who found increased apoB levels as well in normolipidemic survivors of myocardial infarction. However, other variables associated with coronary artery disease such as cigarette smoking, hypertension, and low HDL levels were not studied. In particular, neither the plasma concentration of HDL cholesterol or its major apoprotein, A-I, was measured. It has recently been shown that both parameters of HDL are lower in patients with coronary artery disease (28, 29). Such additional information might even have improved the separation between those with and without coronary disease.

The present study tested the hypothesis that LDL B protein can more accurately delineate coronary disease than other lipid parameters; our findings do indicate a concordance of coronary disease in patients with high levels of B protein but a normal LDL cholesterol. The metabolic basis for these results is not clear. LDL are a complex or series of complex macromolecules occurring within a particular density range and consisting mainly of B protein, phospholipids, free cholesterol and core neutral lipids, cholesterol ester, and triglycerides. LDL cholesterol as an estimate of actual LDL concentration is valid only if the composition of LDL is invariant. However, the proportion of cholesterol in LDL varies in different types of hyperlipidemia (30), in individuals after diet or drug therapy (31, 32), and after myocardial infarction (33). The abnormal LDL composition observed in this study was not due to the effect of lipid-lowering medication or recent myocardial infarction. Nevertheless, the syndrome of hyper*apo*betalipoproteinemia is undoubtedly heterogeneous, and its presence is possibly influenced by a variety of environmental (e.g., dietary) and genetic factors.

Not only is it clear that LDL composition can change or differ in certain situations, but some possible mechanisms for these differences are now becoming apparent. For example, Hammond and Fisher (34) suggested that there are subfractions of LDL, and a lowered ratio of LDL cholesterol to B protein might result from enrichment of a particular type of LDL. Equally possible, the altered composition might indicate an altered or abnormal LDL that has increased atherogenic potential. Another, perhaps more likely, explanation exists. We have previ-

ously shown uptake of cholesteryl ester from LDL across the human splanchnic bed with a corresponding output in VLDL and LDL (27). That cholesteryl ester can be transferred between intact lipoproteins has been directly demonstrated in this and other laboratories (35–38). The cholesterol and, in particular, the cholesteryl ester portion of LDL may be malleable. If so, B protein, a structural component of LDL, may then be reflecting more accurately than cholesterol the amount of LDL in plasma. This suggestion is particularly intriguing because it would link the normocholesterolemic coronary patients with patients who have type II hyperlipoproteinemia. Both have not only coronary disease, but also elevated LDL protein levels. The lipid hypothesis could then be re-examined as a lipoprotein hypothesis; that is, the risk being most proportional to the number of LDL particles and not simply the level of LDL cholesterol. After all, it is the lipoprotein particle that can damage and enter the arterial wall (39) and, thus, initiate the atherogenic process (40). An increased number of LDL particles could arise either from impairment of catabolic processes or from overproduction of VLDL with subsequent increased formation of LDL particles.

The present study does not discriminate between or establish these different hypotheses. It does indicate that levels of LDL B protein may contain additional information about the risk of coronary artery disease and, most importantly, it identifies a new group of individuals with coronary atherosclerosis who would not be recognized by measurement of LDL cholesterol.

We acknowledge the technical assistance of Mrs. Hai Na, the secretarial aid of Miss Wendy Bird, and the encouragement and criticism of Dr. M. McGregor. The present study was supported by the Medical Research Council of Canada (Grant MA 5480) and by Contract HV-1-2158L with the National Heart, Lung and Blood Institute.

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